FORM-PTO-1390 (Rev. 10-96) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 012627-009

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5): 09/142471

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/DE97/00458	07 March 1997	07 March 1996

TITLE OF INVENTION
CONJUGATE FOR MODIFYING INTERACTIONS BETWEEN PROTEINS

APPLICANT(S) FOR DO/EO/US
Stefan ROSE-JOHN

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and the PCT Articles 22 and 39(1).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [X] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [X] has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US)
6. [X] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [ ] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. [X] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [ ] Other items or information:

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.50)	INTERNATIONAL APPLICATION NO. PCT/DE97/00458	ATTORNEY'S DOCKET NUMBER 012627-009

17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)):		
Search Report has been prepared by the EPO or JPO $930		
International preliminary examination fee paid to USPTO (37 CFR 1.482) $720.00		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) $790.00		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO $1070.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) $98.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 930.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	Number Filed	Number Extra	Rate		
Total Claims	11 -20 =	0	X$22.00	$ 930.00	
Independent Claims	1 -3 =	0	X$82.00	$ 930.00	
Multiple dependent claim(s) (if applicable)			+$270.00	$	
TOTAL OF ABOVE CALCULATIONS =				$ 930.00	
Reduction for 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$ 465.00	
SUBTOTAL =				$ 465.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$ 465.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$	
TOTAL FEES ENCLOSED =				$ 465.00	
				Amount to be: refunded	$
				charged	$

a. ☒ A check in the amount of $ 930.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 02-4800 in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-4800. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Teresa Stanek Rea
BURNS, DOANE, SWECKER & MATHIS, L.L.P.
P.O. Box 1404
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SIGNATURE

Teresa Stanek Rea
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30,427
REGISTRATION NUMBER

(10/97)

#3

Patent
Attorney's Docket No. 012627-009

Applicant or Patentee: Stefan Rose-John

Application or Patent No.: 09/142,471

Filed or Issued:

For: CONJUGATE FOR MODIFYING INTERACTIONS BETWEEN PROTEINS

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 C.F.R. §§ 1.9(f) AND 1.27(c)) - SMALL BUSINESS CONCERN

I hereby declare that I am

[ ] the owner of the small business concern identified below:
[ ] an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN ANGEWANDTE GENTECHNOLOGIE SYSTEME GMBH

ADDRESS OF CONCERN RISCHERSTR. 12, D-69123 HEIDELBERG, DE

I hereby declare that the above-identified small business concern qualifies as a small business concern as defined in 13 C.F.R. § 1.21 for purposes of paying reduced fees under Sections 41(a) and 41(b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average, over the previous fiscal year of the concern, of the persons employed on a full-time, part-time, or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention entitled CONJUGATE FOR MODIFYING INTERACTIONS BETWEEN PROTEINS by inventor(s) Stefan Rose-John described in

[ ] the specification filed herewith
[X] Application No. 09/142,471, filed _____.
[ ] Patent No. _____, issued _____.

If the rights held by the above-identified small business concern are not exclusive, each individual, concern, or organization having rights to the invention is listed below,* and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 C.F.R. § 1.9(c), or by any concern that would not qualify as either a small business concern under 37 C.F.R. § 1.9(d) or a nonprofit organization under 37 C.F.R. § 1.9(e).

*NOTE: Separate verified statements are required from each named person, concern, or organization having rights to the invention averring to their status as small entities. (37 C.F.R. § 1.27.)

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Application No. 09/142,471
Attorney's Docket No. 012627-009

NAME _____

ADDRESS _____

[ ] individual [X] small business concern [ ] nonprofit organization

NAME _____

ADDRESS _____

[ ] individual [ ] small business concern [ ] nonprofit organization

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earlier of the issue fee and any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 C.F.R. § 1.28(b).)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code; and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Dr. WALTER G. ROEWEKAMP

TITLE OF PERSON OTHER THAN OWNER Owner

ADDRESS OF PERSON SIGNING Rischerstr. 12, D-69123 Heidelberg

SIGNATURE _____ DATE 10-14-1998

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(12/97)

Patent
Attorney's Docket No. 012627-009

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	
Stefan ROSE-JOHN	Group Art Unit: Unassigned
Application No.: Unassigned (Corresponds to PCT/DE97/00458)	Examiner: Unassigned
International Filing Date: 07 March 1997	
For: CONJUGATE FOR MODIFYING INTERACTIONS BETWEEN PROTEINS	

PRELIMINARY AMENDMENT

BOX PCT
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination, please amend the above-captioned application as follows:

IN THE CLAIMS:

Kindly amend the claims as follows:

Claim 1, line 5, delete "characterized in that" and insert --wherein--;

Claim 2, lines 1-2, delete "characterized in that" and insert --wherein--;

Claim 3, line 1, delete "or 2";

Claim 3, lines 2-3, delete "characterized in that" and insert --wherein--;

Claim 4, lines 1-2, delete "any one of claims 1 to 3, characterized in that" and insert --claim 1, wherein--;

Claim 5, lines 1-2, delete "any one of claims 1 to 3, characterized in that" and insert --claim 1, wherein--;

Claim 6, lines 1-2, delete "any one of claims 1 to 5, characterized in that" and insert --claim 1, wherein--.

Please cancel claim 10.

Kindly add the following new claim:

--11. A method for influencing the interaction between proteins comprising using the conjugate according to claim 1 along with the DNA coding for said conjugate.--

REMARKS

Entry of the foregoing amendment is respectfully requested.

The claims have been amended to eliminate multiple dependency and to place them in better condition for U.S. patent practice.

Should the Examiner have any questions concerning the subject application, a telephone call to the undersigned would be appreciated.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By: _____
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Conjugates for Influencing Interactions Between Proteins

The present invention relates to a conjugate which is suited to influence interactions between proteins, a DNA encoding such a conjugate and the use of the conjugate.

Many processes occurring in an organism are based on interactions between proteins. Examples of such interactions are found in receptors and the ligand binding thereto. However, the interactions between proteins are often unbalanced. This may be due to the fact that individual proteins involved in the interactions are modified, so that their affinity for other proteins which are also involved, is changed. Individual proteins involved in the interactions may also be lacking. This is found e.g. in the case of cells which do not respond to interleukin-6 (IL-6). Such cells have an incomplete interleukin-6 receptor, i.e. this receptor merely comprises the intracellular signal-triggering subunit gp130 but not the extracellular, IL-6-binding subunit (IL-6R).

Many attempts have been made to remedy unbalanced interactions between proteins. For example, this is tried in the case of an incomplete inleukin-6 receptor by administration of IL-6 (50 ng/ml) and soluble IL-6R (sIL-6R) (1280 ng/ml). However, the provision of sIL-6R is expensive and time-consuming, since sIL-6R will only be biologically active if it originates from eukaryotic cells, and the yields therefrom range from 1 to 6 mg sIL-6R/l. Thus, said administration is no suitable means to lastingly remedy the unbalanced interactions in the case of an incomplete interleukin-6 receptor.

Therefore, it is the object of the present invention to provide a product by which unbalanced interactions between proteins can be remedied, particularly in the case of an incomplete interleukin-6 receptor.

According to the invention this is achieved by the subject matters defined in the claims.

Thus, the subject matter of the present invention relates to a conjugate comprising two polypeptides with a mutual affinity, the polypeptides being linked with each other via a linker.

The expression "polypeptides with a mutual affinity" relates to polypeptides of any kind, origin and length, which have an affinity for each other. Two such polypeptides are present in a conjugate according to the invention. One of these polypeptides may be a receptor and the other may be a ligand binding to the receptor. The receptor may be present in the form of its subunit and the functional part thereof, respectively, which are capable to bind the ligand. Likewise, the ligand may be present in the form of its subunit and the functional part thereof, respectively, which are capable to bind the receptor. The receptor is preferably a cytokine receptor, particularly a receptor for lymphokines, monokines, interferons, colony stimulating factors or interleukins. It is especially preferred for the receptor to be an interleukin-6 receptor or a CNTF receptor. The same applies correspondingly to the ligand. It is preferably a cytokine, particularly a lymphokine, monokine, interferon, colony stimulating factor or interleukin. It is especially preferred for the ligand to be a member of the interleukin-6 family, particularly IL-6, IL-11, CNTF, OSM, LIF or CT-1. The receptor and the ligand may comprise wild-type sequences or sequences differing therefrom by one or several nucleotides. As a result, the receptor and the ligand may have improved and/or new properties. For example, improved properties may be represented by the fact that the bond between receptor and ligand is improved. For example, new properties may be represented by the fact that the ligand shows a behavior modified with respect to proteins with which it reacts after binding to the receptor. For example, IL-6 may be modified to the effect that it binds more strongly to the IL-6 receptor, but can no longer

activate the protein gp130. In such a case, IL-6 comprises preferably the sequence of fig. 3 or fragments thereof. The above statements made on a modification of the wild-type sequence of a receptor and a ligand, respectively, apply correspondingly to the other subunits and functional parts thereof, which contribute to a mutual bond.

The expression "linker" refers to linkers of any kind, which are suited to bind polypeptides. Examples of such linkers are bifunctional, chemical cross-linkers, e.g. DPDPB. Moreover, the linker may be a disulfide bridge formed by both polypeptides. In addition, the linker may be a polypeptide.

In a preferred embodiment, an above conjugate is a fusion polypeptide. It may contain the two polypeptides which have a mutual affinity and are fused to each other, and the linker may represent a disulfide bridge formed by the two polypeptides. The linker is preferably a polypeptide which binds the two other polypeptides with each other. Examples of the latter fusion polypeptide are indicated in figs. 1 and 2. These fusion polypeptides comprise a human sIL-6R polypeptide, i.e. the extracellular subunit of an interleukin-6 receptor, and a human IL-6 polypeptide, the polypeptides being linked with each other via differing polypeptide linkers. These fusion polypeptides are referred to as H-IL-6. A variation of H-IL-6 which only contains the amino acids Pro 114 to Ala 323 of the sIL-6R polypeptide, is also provided. Furthermore, a variation of H-IL-6 is provided which comprises amino acids 113 to 323 of the sIL-6R polypeptide and amino acids 29 to 212 of the IL-6 polypeptide. In addition, a fusion polypeptide H-IL-6 is provided whose IL-6 polypeptide comprises the sequence of fig. 3. The sIL-6R polypeptide of this fusion polypeptide comprises a complete sequence and the sequence between amino acids 113 (114) to 323 of an sIL-6R polypeptide, respectively. Besides, a fusion polypeptide is provided which comprises the extracellular subunit of a human CNTF

receptor and human CNTF, both polypeptides being linked with each other via a polypeptide linker.

A further subject matter of the present invention relates to a DNA coding for an above fusion polypeptide. The DNA codes preferably for a fusion polypeptide in which both polypeptides with a mutual affinity are linked with each other via a polypeptide linker. An example of the latter DNA is indicated in fig. 1. This DNA was deposited with the DSM (*Deutsche Sammlung von Mikroorganismen und Zellkulturen* [German-type collection of micro organisms and cell cultures]) as CDM8-H-IL-6 under DSM 10549 on February 27, 1996.

A DNA according to the invention can be present in a vector and expression vector, respectively. A person skilled in the art is familiar with examples thereof. In the case of an expression vector for E. coli these are e.g. pGEMEX, pUC derivatives, pGEX-2T, pET3b and pQE-8. For the expression in yeast, e.g. pY100, Ycpad1 and vectors for Pichia pastoris have to be mentioned, the latter being preferred, while for the expression in animal cells, which may be present within an organism or outside thereof, e.g. pKCR, pEFBOS, pCEV4 and pCDM8 have to be indicated, the latter being preferred. The baculovirus expression vector pAcSGHisNT-A is especially suitable for the expression in insect cells. The person skilled in the art will take into consideration that for the expression of a DNA according to the invention, which contains sIL-6R sequences, it is advisable to use vectors which enable an expression in eukaryotic cells.

However, the person skilled in the art is familiar with suitable cells to express a DNA according to the invention, which is present in an expression vector. Examples of such cells comprise the E. coli strains HB101, DH1, x1776, JM101, JM109, BL21 and SG13009, the yeast strain Saccharomyces cerevisiae and Pichia pastoris, the latter being preferred, the animal cells L, 3T3, FM3A, CHO, Vero, HeLa and COS, the latter being preferred, as well as the insect cells sf9.

The person skilled in the art also knows how to insert a DNA according to the invention in an expression vector. In addition, he knows conditions of transforming cells and transfecting cells, respectively, and then cultivating them. He is also familiar with processes of isolating and purifying the fusion polypeptide expressed by the DNA according to the invention.

A further subject matter of the present invention relates to an antibody directed against an above fusion polypeptide. Such an antibody can be prepared by common methods. It may be polyclonal and monoclonal, respectively. For its preparation it is favorable to immunize animals - particularly rabbits or chickens for a polyclonal antibody and mice for a monoclonal antibody - with an above fusion polypeptide. Further "boosters" of the animals can be effected with the same fusion polypeptide. The polyclonal antibody may then be obtained from the animal serum and egg yolk, respectively. For the preparation of the monoclonal antibody, animal spleen cells are fused with myeloma cells.

By means of the present invention it is possible to influence the interactions between proteins. This can be done by administering conjugates according to the invention and by using DNA according to the invention in a gene therapy. In particular, the unbalanced interactions can be remedied in the case of an incomplete interleukin-6 receptor. The present invention distinguishes itself in that it can be used in a cost-effective manner. This manifests itself particularly in the administration of conjugates according to the invention to influence the unbalanced interactions in the case of an incomplete interleukin-6 receptor.

Furthermore, the present invention is suited for the *ex vivo* expansion of stem cells, particularly human stem cells. In this connection, it is especially remarkable that it is

possible by means of a conjugate H-IL-6 according to the invention to obtain more stem cell colonies in the soft agar than possible with the individual components IL-6 and sIL-6R. Thus, the present invention also represents an important contribution to the well-calculated influence of blood cell formation.

By means of a fusion polypeptide H-IL-6 which comprises the sequence of fig. 3 as IL-6 polypeptide, the present invention also provides a product which is suitable as IL-6 receptor antagonist. Such a product is of great therapeutic significance.

The carrying-out of the present invention can be controlled by the antibodies according to the invention.

Brief description of the drawing

Fig. 1 shows the amino acid (DNA) sequence of a fusion polypeptide H-IL-6 according to the invention. Sequences for the restriction enzyme SalI (GTCGAC), the signal peptide (MLAVGCALLAALLAAPGAA) and the linker (RGGGGSGGGGSGGGGSVE) are indicated. The linker links the COOH terminus of human sIL-6R with the NH_2 terminus of human IL-6.

Fig. 2 shows the amino acid (DNA) sequence of a fusion polypeptide H-IL-6 according to the invention. Sequences for the restriction enzyme SalI (GTCGAC), the signal peptide (MLAVGCALLAALLAAPGAA) and the linker (RGGGGSGGGGSVE) are indicated. The linker links the COOH terminus of human sIL-6R with the NH_2 terminus of human IL-6.

Fig. 3 shows the amino acid sequence of the IL-6 polypeptide present in a fusion polypeptide H-IL-6 according to the invention.

Fig. 4 shows the expansion and colony forming capacities of a fusion polypeptide H-IL-6 according to the invention.

The invention is explained by the below examples.

Example 1: Preparation of a DNA according to the invention

The DNA of fig. 1 was prepared. For this purpose, human IL-6R cDNA (Schooltink et al., Biochem. J. (1991) 277, 659-664) was used. This cDNA was cloned into the expression plasmid pCDM8 via restriction site Xho I (Müllberg et al., Eur. J. Immunol. (1993) 23, 473-480). By means of a polymerase chain reaction (PCR), an sIL-6R fragment was generated by using the primer (1) (pCDM8 5' primer: 5' TAATACGACTCACTATAGGG3') and primer (2) (sIL-6R 3' primer: 5'CCGCTCGAGCTGGAGGACTC-CTGGA 3') under normal conditions. After being cut with restriction enzymes Hind III and Xho I, this fragment was cloned into the open plasmid pCDM8. The plasmid pCDM8-sIL-6R formed. Thereafter, a second PCR reaction was carried out with IL-6 cDNA which had also been cloned into the expression plasmid pCDM8 by using Xho I. The primers (3) (IL-6-5' primer: 5' CGGCTCGAGCCAGTACCCCCAGGAGAA3') and primer (4) (pCDM8 3' primer: 5'CCACAGAAGTAAGGTTCCTT3') were used. The PCR product was cut with restriction enzymes Xho I and Not I and cloned into plasmid pCDM8-sIL-6R. The plasmid pCDM8-sIL-6R-IL-6 formed. Thereafter, a synthetic linker was prepared which consisted of two oligonucleotides: primer (5) (5'TCGAGGAGGTGGAGGTTCTGGAGGTGGAGGTTCTGGAGGTGGAGGTTCTG3') and primer (6) (5'TCGACAGAACCTCCACCTCCAGAACCTCCACCTCCAGAACCTCCA-CCTCC3'). Oligonucleotides (5) and (6) were combined according to standard methods into a double strand and then cloned into the plasmid pCDM8-sIL-R-IL-6 digested by the restriction enzyme Xho I. The plasmid pCDM8-H-IL-6 formed.

Example 2: Preparation of a DNA according to the invention

The DNA of fig. 2 was prepared. For this purpose, the steps as described in Example 1 were carried out. However, the following primers were used as primers (5) and (6): primer (5) (5'TCGAGGAGGTGGAGGTTCTGGAGGTGGAGGTTCTG3') and primer (6) (5'TCGACAGAACCTCCACCTCCAGAACCTCCACCTCC3'). The plasmid pCDM8-H-IL-6-(2) was obtained.

Example 3: Expression of a fusion polypeptide according to the invention

COS-7 cells were transfected with pCDM8-H-IL-6 of Example 1 and pCDM8-H-IL-6(2) of Example 2, respectively, by means of electroporation. 10^7 COS-7 cells were electroporated with 20 μg plasmid by means of a gene pulser (Bio-Rad) at 960 μF and 230 V. 48 h after the transfection, the cells were radioactively labeled metabolically using [^{35}S] cysteine/methionine for 4 h and incubated with amino acids which were not labeled radioactively for 2 h. The supernatant from cell lysate and cell supernatant was immunoprecipitated according to standard methods (Müllberg et al., Eur. J. Immunol. (1993) 23, 473-480) using an anti-IL-6 antibody and made visible by autoradiography after SDS gel electrophoresis. Transfected COS-7 cells secreted a 70-75 kDa protein which was recognized by an anti-IL-6 antibody and was not formed by non-transfected cells.

Supernatants of transfected COS-7 cells were separated by SDS gel electrophoresis, transferred to nitrocellulose and detected with an anti-IL-6 antibody. Again, transfected COS-7 cells expressed a 70-75 kDa protein which was recognized by an anti-IL-6 antibody.

Supernatants of transfected COS-7 cells were investigated by means of a commercial ELISA for IL-6 (CLB, Amsterdam, Netherlands) and sIL-6R (Seromed, Gießen, FRG). H-IL-6 was

detected by means of both ELISAs. The concentration of H-IL-6 in the cell supernatant was about 1 µg/ml.

Example 4: Stimulation of the haptoglobin expression by a fusion polypeptide according to the invention

The human hepatoma cell lines HepG2, HepG2-IL-6 and HepG2-PDI were used.

HepG2 cells (ATCC HB 8065) are stimulated to express haptoglobin by IL-6, but not by sIL-6R.

HepG2-IL-6 cells were obtained by stable transfection of HepG2 cells with a human IL-6 expression plasmid. On account of the IL-6 expression these cells down-regulate endogenous IL-6R and thus express no IL-6R. HepG2-IL-6 cells are not stimulated to express haptoglobin by IL-6, but by sIL-6R.

HepG2-PDI cells were obtained by stable transfection of HepG2 cells with a human IL-6 expression plasmid. For this purpose, the expression plasmid included an IL-6 cDNA by which the expressed IL-6 protein included a COOH-terminal retention signal for the endoplasmic reticulum (ER). As a result, these cells did not only retain the expressed IL-6 but also IL-6R in the ER. In contrast to HepG2-IL-6 cells, HepG2-PDI cells do not secrete IL-6 and can only be stimulated to express haptoglobin by a combination of IL-6 and sIL-6R.

The above hepatoma cell lines were cultivated under standard conditions in 96-well cell culture plates (Rose-John et al., J. Biol. Chem. 268 (1993), 22084-22091). The cells were stimulated with IL-6, sIL-6R, IL-6 + sIL-6R and cell supernatants, respectively, which originated from COS-7 cells of Example 3, transfected with pCDM8-H-IL-6, pCDM8-H-IL-6(2) and pCDM8, respectively, for 18 h. The cell supernatant was collected and the haptoglobin concentration

in the supernatant was determined by means of ELISA (cf. Table 1).

Table 1

Stimulation of the haptoglobin expression

	IL-6	sIL-6R	IL-6 + sIL-6R	H-IL-6	control
HepG2	+	-	+ +	+ + +	-
HepG2-IL-6	-	+ +	+ +	+ + +	-
HepG2-PDI	-	-	+ +	+ + +	-

It showed that a fusion polypeptide according to the invention, H-IL-6, is capable of stimulating the expression of haptoglobin in cells, i.e. of influencing the interactions between proteins.

Example 5: Expansion and colony formation of human CD34$^+$ cells by a fusion polypeptide according to the invention

Cells which express the surface marker CD34 were isolated from human bone marrow and blood of patients whose stem cells had been mobilized by injection of G-CSF, respectively. 6000 of these cells were plated in 3 ml medium in cell culture vessels. After two weeks it turned out that an incubation of the cells with cytokines SCF, IL-3 and H-IL-6 (fusion polypeptide according to the invention) as well as SCF, IL-3 and IL-6 caused strong proliferation. 1000 cells of the resulting cells were plated into new cell culture vessels. After two weeks in a standardized colony induction experiment, the cells treated with SCF, IL-3 and H-IL-6 were capable of forming about three times more colonies than cells treated with SCF, IL-3 and IL-6.

This result shows that cells stimulated by a fusion polypeptide H-IL-6 according to the invention have a greater

colony-forming potential than cells stimulated by Il-6 (cf. fig. 4).

Article 34

Amended **Claims**

1. A conjugate comprising two polypeptides with a mutual affinity, one polypeptide being a cytokine receptor and the other polypeptide being a cytokine as ligand and the polypeptides being linked with each other via a linker, characterized in that the linker is a disulfide bridge formed by two polypeptides or a polypeptide.

2. The conjugate according to claim 1, characterized in that the receptor is present in the form of its subunit binding the ligand.

3. The conjugate according to claim 1 or 2, characterized in that the ligand is present in the form of its subunit binding the receptor.

4. The conjugate according to any one of claims 1 to 3, characterized in that the cytokine receptor is an IL-6 receptor and the cytokine is an IL-6.

5. The conjugate according to any one of claims 1 to 3, characterized in that the cytokine receptor is a CNTF receptor and the cytokine is a CNTF.

6. The conjugate according to any one of claims 1 to 5, characterized in that the conjugate is a fusion polypeptide.

7. DNA coding for the conjugate according to claim 6.

8. An expression plasmid comprising the DNA according to claim 7.

9. A transformant containing the expression plasmid according to claim 8.

10. Use of the conjugate according to any one of claims 1 to 6 and the DNA according to claim 7 for influencing the interactions between proteins.

Abstract of the Disclosure

The present invention concerns a conjugate comprising two polypeptides with a mutual affinity, the polypeptides being connected with each other via a linker. This invention also concerns the use of such a conjugate to influence interactions between proteins.

```
1       GTCGACGCATGGAGTGGTAGCCGAGGAGGAAGC ATG CTG GCC GTC GGC TGC GCG CTG CTG GCT   63
1                                          M   L   A   V   G   C   A   L   L   A   10

64  GCC CTG CTG GCC GCG CCG GGA GCG GCG CTG GCC CCA AGG CGC TGC CCT GCG CAG GAG GTG  123
11  A   L   L   A   A   P   G   A   A   L   A   P   R   R   C   P   A   Q   E   V   30

124 GCA AGA GGC GTG CTG ACC AGT CTG CCA GGA GAC AGC GTG ACT CTG ACC TGC CCG GGG GTA  183
31  A   R   G   V   L   T   S   L   P   G   D   S   V   T   L   T   C   P   G   V   50

184 GAG CCG GAA GAC AAT GCC ACT GTT CAC TGG GTG CTC AGG AAG CCG GCT GCA GGC TCC CAC  243
51  E   P   E   D   N   A   T   V   H   W   V   L   R   K   P   A   A   G   S   H   70

244 CCC AGC AGA TGG GCT GGC ATG GGA AGG AGG CTG CTG CTG AGG TCG GTG CAG CTC CAC GAC  303
71  P   S   R   W   A   G   M   G   R   R   L   L   L   R   S   V   Q   L   H   D   90

304 TCT GGA AAC TAT TCA TGC TAC CGG GCC GGC CGC CCA GCT GGG ACT GTG CAC TTG CTG GTG  363
91  S   G   N   Y   S   C   Y   R   A   G   R   P   A   G   T   V   H   L   L   V   110

364 GAT GTT CCC CCC GAG GAG CCC CAG CTC TCC TGC TTC CGG AAG AGC CCC CTC AGC AAT GTT  423
111 D   V   P   P   E   E   P   Q   L   S   C   F   R   K   S   P   L   S   N   V   130

424 GTT TGT GAG TGG GGT CCT CGG AGC ACC CCA TCC CTG ACG ACA AAG GCT GTG CTC TTG GTG  483
131 V   C   E   W   G   P   R   S   T   P   S   L   T   T   K   A   V   L   L   V   150

484 AGG AAG TTT CAG AAC AGT CCG GCC GAA GAC TTC CAG GAG CCG TGC CAG TAT TCC CAG GAG  543
151 R   K   F   Q   N   S   P   A   E   D   F   Q   E   P   C   Q   Y   S   Q   E   170

544 TCC CAG AAG TTC TCC TGC CAG TTA GCA GTC CCG GAG GGA GAC AGC TCT TTC TAC ATA GTG  603
171 S   Q   K   F   S   C   Q   L   A   V   P   E   G   D   S   S   F   Y   I   V   190

604 TCC ATG TGC GTC GCC AGT AGT GTC GGG AGC AAG TTC AGC AAA ACT CAA ACC TTT CAG GGT  663
191 S   M   C   V   A   S   S   V   G   S   K   F   S   K   T   Q   T   F   Q   G   210

664 TGT GGA ATC TTG CAG CCT GAT CCG CCT GCC AAC ATC ACA GTC ACT GCC GTG GCC AGA AAC  723
211 C   G   I   L   Q   P   D   P   P   A   N   I   T   V   T   A   V   A   R   N   230

724 CCC CGC TGG CTC AGT GTC ACC TGG CAA GAC CCC CAC TCC TGG AAC TCA TCT TTC TAC AGA  783
231 P   R   W   L   S   V   T   W   Q   D   P   H   S   W   N   S   S   F   Y   R   250

784 CTA CGG TTT GAG CTC AGA TAT CGG GCT GAA CGG TCA AAG ACA TTC ACA ACA TGG ATG GTC  843
251 L   R   F   E   L   R   Y   R   A   E   R   S   K   T   F   T   T   W   M   V   270

844 AAG GAC CTC CAG CAT CAC TGT GTC ATC CAC GAC GCC TGG AGC GGC CTG AGG CAC GTG GTG  903
271 K   D   L   Q   H   H   C   V   I   H   D   A   W   S   G   L   R   H   V   V   290

904 CAG CTT CGT GCC CAG GAG GAG TTC GGG CAA GGC GAG TGG AGC GAG TGG AGC CCG GAG GCC  963
291 Q   L   R   A   Q   E   E   F   G   Q   G   E   W   S   E   W   S   P   E   A   310

964 ATG GGC ACG CCT TGG ACA GAA TCC AGG AGT CCT CCA GCT CGA GGA GGT GGA GGT TCT GGA 1023
311 M   G   T   P   W   T   E   S   R   S   P   P   A   R   G   G   G   G   S   G   330

1024 GGT GGA GGT TCT GGA GGT GGA GGT TCT GTC GAG CCA GTA CCC CCA GGA GAA GAT TCC AAA 1083
331  G   G   G   S   G   G   G   G   S   V   E   P   V   P   P   G   E   D   S   K   350

1084 GAT GTA GCC GCC CCA CAC AGA CAG CCA CTC ACC TCT TCA GAA CGA ATT GAC AAA CAA ATT 1143
351  D   V   A   A   P   H   R   Q   P   L   T   S   S   E   R   I   D   K   Q   I   370

1144 CGG TAC ATC CTC GAC GGC ATC TCA GCC CTG AGA AAG GAG ACA TGT AAC AAG AGT AAC ATG 1203
371  R   Y   I   L   D   G   I   S   A   L   R   K   E   T   C   N   K   S   N   M   390

1204 TGT GAA AGC AGC AAA GAG GCA CTG GCA GAA AAC AAC CTG AAC CTT CCA AAG ATG GCT GAA 1263
391  C   E   S   S   K   E   A   L   A   E   N   N   L   N   L   P   K   M   A   E   410

1264 AAA GAT GGA TGC TTC CAA TCT GGA TTC AAT GAG GAG ACT TGC CTG GTG AAA ATC ATC ACT 1323
411  K   D   G   C   F   Q   S   G   F   N   E   E   T   C   L   V   K   I   I   T   430

1324 GGT CTT TTG GAG TTT GAG GTA TAC CTA GAG TAC CTC CAG AAC AGA TTT GAG AGT AGT GAG 1383
431  G   L   L   E   F   E   V   Y   L   E   Y   L   Q   N   R   F   E   S   S   E   450

1384 GAA CAA GCC AGA GCT GTG CAG ATG AGT ACA AAA GTC CTG ATC CAG TTC CTG CAG AAA AAG 1443
451  E   Q   A   R   A   V   Q   M   S   T   K   V   L   I   Q   F   L   Q   K   K   470

1444 GCA AAG AAT CTA GAT GCA ATA ACC ACC CCT GAC CCA ACC ACA AAT GCC AGC CTG CTG ACG 1503
471  A   K   N   L   D   A   I   T   T   P   D   P   T   T   N   A   S   L   L   T   490

1504 AAG CTG CAG GCA CAG AAC CAG TGG CTG CAG GAC ATG ACA ACT CAT CTC ATT CTG CGC AGC 1563
491  K   L   Q   A   Q   N   Q   W   L   Q   D   M   T   T   H   L   I   L   R   S   510

1564 TTT AAG GAG TTC CTG CAG TCC AGC CTG AGG GCT CTT CGG CAA ATG TAG CATGGGCACCGTCGAC 1627
511  F   K   E   F   L   Q   S   S   L   R   A   L   R   Q   M   *                   525
```

FIG. 1

```
   1 GTCGACGC ATG GAG TGG TAG CCGAGGAGGAAGC ATG CTG GCC GTC GGC TGC GCG CTG CTG GCT   63
   1                                        M  L  A  V  G  C  A  L  L  A            10

  64 GCC CTG CTG GCC GCG CCG GGA GCG GCG CTG GCC CCA AGG CGC TGC CCT GCG CAG GAG GTG  123
  11 A  L  L  A  A  P  G  A  A  L  A  P  R  R  C  P  A  Q  E  V                    30

 124 GCA AGA GGC GTG CTG ACC AGT CTG CCA GGA GAC AGC GTG ACT CTG ACC TGC CCG GGG GTA  183
  31 A  R  G  V  L  T  S  L  P  G  D  S  V  T  L  T  C  P  G  V                    50

 184 GAG CCG GAA GAC AAT GCC ACT GTT CAC TGG GTG CTC AGG AAG CCG GCT GCA GGC TCC CAC  243
  51 E  P  E  D  N  A  T  V  H  W  V  L  R  K  P  A  A  G  S  H                    70

 244 CCC AGC AGA TGG GCT GGC ATG GGA AGG AGG CTG CTG CTG AGG TCG GTG CAG CTC CAC GAC  303
  71 P  S  R  W  A  G  M  G  R  R  L  L  L  R  S  V  Q  L  H  D                    90

 304 TCT GGA AAC TAT TCA TGC TAC CGG GCC GGC CGC CCA GCT GGG ACT GTG CAC TTG CTG GTG  363
  91 S  G  N  Y  S  C  Y  R  A  G  R  P  A  G  T  V  H  L  L  V                   110

 364 GAT GTT CCC CCC GAG GAG CCC CAG CTC TCC TGC TTC CGG AAG AGC CCC CTC AGC AAT GTT  423
 111 D  V  P  P  E  E  P  Q  L  S  C  F  R  K  S  P  L  S  N  V                   130

 424 GTT TGT GAG TGG GGT CCT CGG AGC ACC CCA TCC CTG ACG ACA AAG GCT GTG CTC TTG GTG  483
 131 V  C  E  W  G  P  R  S  T  P  S  L  T  T  K  A  V  L  L  V                   150

 484 AGG AAG TTT CAG AAC AGT CCG GCC GAA GAC TTC CAG GAG CCG TGC CAG TAT TCC CAG GAG  543
 151 R  K  F  Q  N  S  P  A  E  D  F  Q  E  P  C  Q  Y  S  Q  E                   170

 544 TCC CAG AAG TTC TCC TGC CAG TTA GCA GTC CCG GAG GGA GAC AGC TCT TTC TAC ATA GTG  603
 171 S  Q  K  F  S  C  Q  L  A  V  P  E  G  D  S  S  F  Y  I  V                   190

 604 TCC ATG TGC GTC GCC AGT AGT GTC GGG AGC AAG TTC AGC AAA ACT CAA ACC TTT CAG GGT  663
 191 S  M  C  V  A  S  S  V  G  S  K  F  S  K  T  Q  T  F  Q  G                   210

 664 TGT GGA ATC TTG CAG CCT GAT CCG CCT GCC AAC ATC ACA GTC ACT GCC GTG GCC AGA AAC  723
 211 C  G  I  L  Q  P  D  P  P  A  N  I  T  V  T  A  V  A  R  N                   230

 724 CCC CGC TGG CTC AGT GTC ACC TGG CAA GAC CCC CAC TCC TGG AAC TCA TCT TTC TAC AGA  783
 231 P  R  W  L  S  V  T  W  Q  D  P  H  S  W  N  S  S  F  Y  R                   250

 784 CTA CGG TTT GAG CTC AGA TAT CGG GCT GAA CGG TCA AAG ACA TTC ACA ACA TGG ATG GTC  843
 251 L  R  F  E  L  R  Y  R  A  E  R  S  K  T  F  T  T  W  M  V                   270

 844 AAG GAC CTC CAG CAT CAC TGT GTC ATC CAC GAC GCC TGG AGC GGC CTG AGG CAC GTG GTG  903
 271 K  D  L  Q  H  H  C  V  I  H  D  A  W  S  G  L  R  H  V  V                   290

 904 CAG CTT CGT GCC CAG GAG GAG TTC GGG CAA GGC GAG TGG AGC GAG TGG AGC CCG GAG GCC  963
 291 Q  L  R  A  Q  E  E  F  G  Q  G  E  W  S  E  W  S  P  E  A                   310

 964 ATG GGC ACG CCT TGG ACA GAA TCC AGG AGT CCT CCA GCT CGA GGA GGT GGA GGT TCT GGA 1023
 311 M  G  T  P  W  T  E  S  R  S  P  P  A  R  G  G  G  G  S  G                   330

1024 GGT GGA GGT TCT GTC GAG CCA GTA CCC CCA GGA GAA GAT TCC AAA GAT GTA GCC GCC CCA 1083
 331 G  G  G  S  V  E  P  V  P  P  G  E  D  S  K  D  V  A  A  P                   350

1084 CAC AGA CAG CCA CTC ACC TCT TCA GAA CGA ATT GAC AAA CAA ATT CGG TAC ATC CTC GAC 1143
 351 H  R  Q  P  L  T  S  S  E  R  I  D  K  Q  I  R  Y  I  L  D                   370

1144 GGC ATC TCA GCC CTG AGA AAG GAG ACA TGT AAC AAG AGT AAC ATG TGT GAA AGC AGC AAA 1203
 371 G  I  S  A  L  R  K  E  T  C  N  K  S  N  M  C  E  S  S  K                   390

1204 GAG GCA CTG GCA GAA AAC AAC CTG AAC CTT CCA AAG ATG GCT GAA AAA GAT GGA TGC TTC 1263
 391 E  A  L  A  E  N  N  L  N  L  P  K  M  A  E  K  D  G  C  F                   410

1264 CAA TCT GGA TTC AAT GAG GAG ACT TGC CTG GTG AAA ATC ATC ACT GGT CTT TTG GAG TTT 1323
 411 Q  S  G  F  N  E  E  T  C  L  V  K  I  I  T  G  L  L  E  F                   430

1324 GAG GTA TAC CTA GAG TAC CTC CAG AAC AGA TTT GAG AGT AGT GAG GAA CAA GCC AGA GCT 1383
 431 E  V  Y  L  E  Y  L  Q  N  R  F  E  S  S  E  E  Q  A  R  A                   450

1384 GTG CAG ATG AGT ACA AAA GTC CTG ATC CAG TTC CTG CAG AAA AAG GCA AAG AAT CTA GAT 1443
 451 V  Q  M  S  T  K  V  L  I  Q  F  L  Q  K  K  A  K  N  L  D                   470

1444 GCA ATA ACC ACC CCT GAC CCA ACC ACA AAT GCC AGC CTG CTG ACG AAG CTG CAG GCA CAG 1503
 471 A  I  T  T  P  D  P  T  T  N  A  S  L  L  T  K  L  Q  A  Q                   490

1504 AAC CAG TGG CTG CAG GAC ATG ACA ACT CAT CTC ATT CTG CGC AGC TTT AAG GAG TTC CTG 1563
 491 N  Q  W  L  Q  D  M  T  T  H  L  I  L  R  S  F  K  E  F  L                   510

1564 CAG TCC AGC CTG AGG GCT CTT CGG CAA ATG TAG C ATG GGC ACC GTC GAC               1612
 511 Q  S  S  L  R  A  L  R  Q  M  *                                               520
```

FIG. 2

Met Asn Ser Phe Ser Thr Ser Ala Phe Gly Pro Val Ala Phe Ser Leu
Gly Leu Leu Leu Val Leu Pro Ala Ala Phe Pro Ala Pro Val Pro Pro
1 5 10 15 20
Gly Glu Asp Ser Lys Asp Val Ala Ala Pro His Arg Gln Pro Leu Thr
Ser Ser Glu Arg Ile Asp Lys Gln Ile Arg Tyr Ile Leu Asp Gly Ile
25 30 35
Ser Ala Leu Arg Lys Glu Thr Cys Asn Lys Ser Asn Met Cys Glu Ser
40 45 50
Ser Pro Glu Ala Leu Ala Glu Asn Asn Leu Asn Leu Pro Lys Met Ala
55 60 65
Glu Lys Asp Gly Cys Phe Gln Ser Gly Phe Asn Glu Glu Thr Cys Leu
70 75 80
Val Lys Ile Ile Thr Gly Leu Leu Glu Phe Glu Val Tyr Leu Glu Tyr
85 90 95 100
Leu Gln Asn Arg Phe Glu Ser Ser Glu Glu Gln Ala Arg Ala Val Gln
105 110 115
Met Ser Thr Lys Val Leu Ile Gln Phe Leu Gln Lys Lys Ala Lys Asn
120 125 130
Leu Asp Ala Ile Thr Thr Pro Asp Pro Thr Thr Asn Ala Ser Leu Leu
135 140 145
Thr Lys Leu Gln Ala Gln Asn Gln Trp Leu Glu Asp Met Pro Thr His
150 155 160
Leu Ile Leu Arg Ser Leu Lys Glu Phe Leu Gln Arg Ser Leu Arg Ala
165 170 175 180
Leu Arg Gln Met
184

FIG. 3

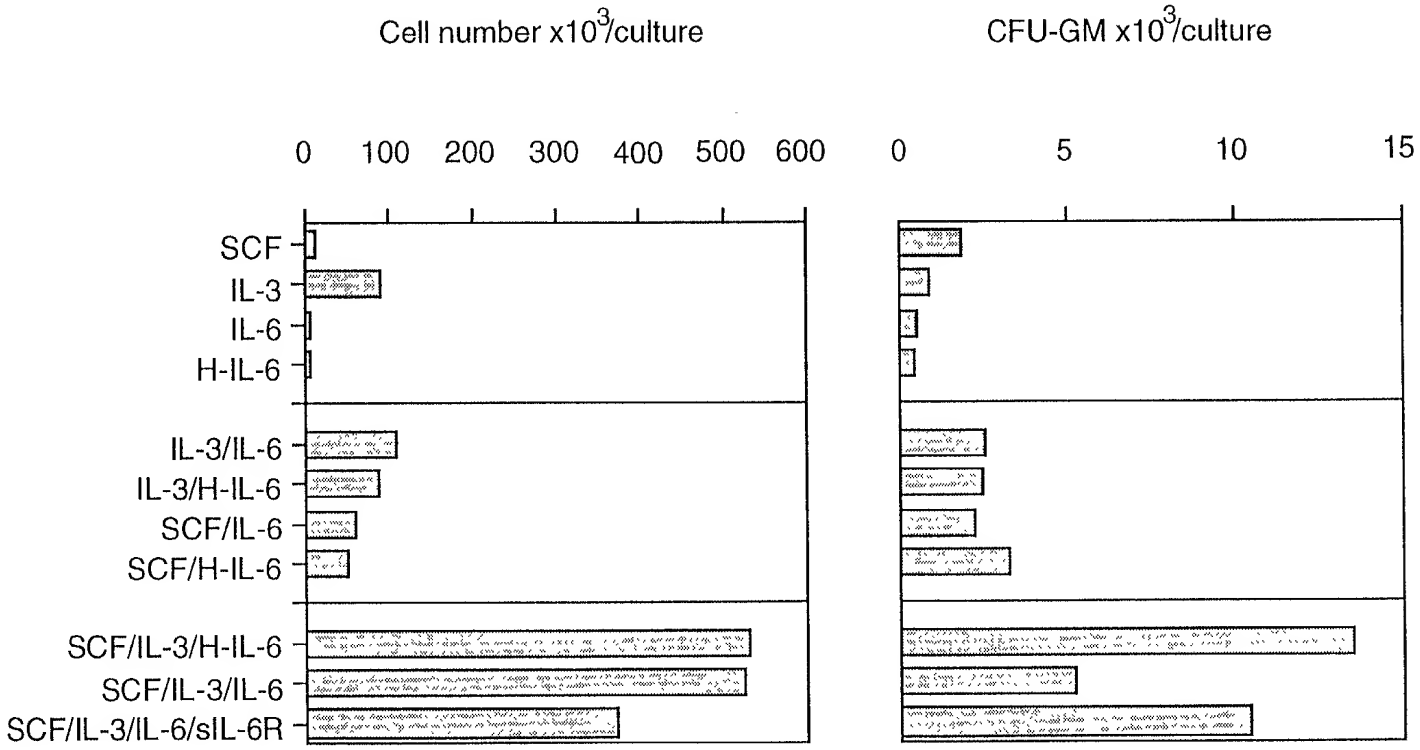

FIG. 4

#3

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (Includes Reference to Provisional and PCT International Applications)	ATTORNEY'S DOCKET NUMBER 012627-009

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name;
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

CONJUGATE FOR MODIFYING INTERACTIONS BETWEEN PROTEINS

the specification of which (check only one item below):

☐ is attached hereto.

☐ was filed as United States application

Number _____

on _____

and was amended

on _____ (if applicable).

☒ was filed as PCT international application

Number PCT/DE97/00458

on 07 March 1997

and was amended under PCT Article 19

on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(e) of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. §119:

COUNTRY (if PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. §119
DE	196 08 813.5	07 March 1996	X Yes __No
			__Yes __No
			__Yes __No
			__Yes __No
			__Yes __No

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

_____ _____
(Application Number) (Filing Date)

_____ _____
(Application Number) (Filing Date)

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (CONTINUED) (Includes Reference to Provisional and PCT International Applications)	ATTORNEY'S DOCKET NO. 012627-009

I hereby claim the benefit under Title 35, United States Code, §120 of any United States applications(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose to the Office all information known to me to be material to the patentability as defined in Title 37, Code of Federal Regulations §1.56, which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application:

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120:

U.S. APPLICATIONS		STATUS (check one)		
U.S. APPLICATION NUMBER	U.S. FILING DATE	PATENTED	PENDING	ABANDONED

PCT APPLICATIONS DESIGNATING THE U.S.					
PCT APPLICATION NO.	PCT FILING DATE	U.S. APPLICATION NUMBERS ASSIGNED (if any)			

I hereby appoint the following attorneys and agent(s) to prosecute said application and to transact all business in the Patent and Trademark Office connected therewith and to file, prosecute and to transact all business in connection with international applications directed to said invention:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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FULL NAME OF SOLE OR FIRST INVENTOR Stefan ROSE-JOHN	SIGNATURE	DATE 10-20-99
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POST OFFICE ADDRESS Obere Zahlbacherstrasse 63, D-55101 Mainz, Germany		
FULL NAME OF SECOND JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		
FULL NAME OF THIRD JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		
FULL NAME OF FOURTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		
FULL NAME OF FIFTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		
FULL NAME OF SIXTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		
FULL NAME OF SEVENTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		
FULL NAME OF EIGHTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		
FULL NAME OF NINTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		